Title

A preferential loss of GABAergic, symmetric synapses in epileptic foci: a quantitative ultrastructural analysis of monkey neocortex.

Journal

The Journal of neuroscience : the official journal of the Society for Neuroscience, 2(12)

ISSN

0270-6474

Authors

Ribak, CE
Bradburne, RM
Harris, AB

Publication Date

1982-12-01

DOI

10.1523/jneurosci.02-12-01725.1982

0270-6474/82/0212-1725$02.00/0

Printed in U.S.A.

The Journal of Neuroscience
Vol. 2, No. 12, pp. 1725-1735
December 1982

A PREFERENTIAL LOSS OF GABAERGIC, SYMMETRIC SYNAPSES IN EPILEPTIC FOCI: A QUANTITATIVE ULTRASTRUCTURAL ANALYSIS OF MONKEY NEOCORTEX[1]

CHARLES E. RIBAK,[2] R. MARK BRADBURNE, AND A. BASIL HARRIS*

*Department of Anatomy, University of California, Irvine, Irvine, California 92717 and *Department of Neurological Surgery, University of Washington, Seattle, Washington 98195*

Received December 2, 1981; Revised May 11, 1982; Accepted June 10, 1982

Abstract

Previous immunocytochemical results from five monkeys with cortical focal epilepsy produced by alumina gel showed a severe decrease at seizure foci of axon terminals that contained glutamic acid decarboxylase (GAD), the synthesizing enzyme for the inhibitory neurotrasmitter, GABA. These data indicated a functional loss of GABAergic terminals but did not show: (*1*) whether this loss was caused by GABAergic nerve terminal degeneration or by a lack of GAD immunoreactivity within these terminals and (*2*) if this loss of GABAergic terminals was selective for only this terminal type. To resolve these issues, cortical tissue from three of the five monkeys used in the previous study was reexamined using electron microscopy, and a quantitative morphological analysis of cortical structures was made to compare profiles of terminals and glia in the nonepileptic cortex with those in the focus and parafocus.

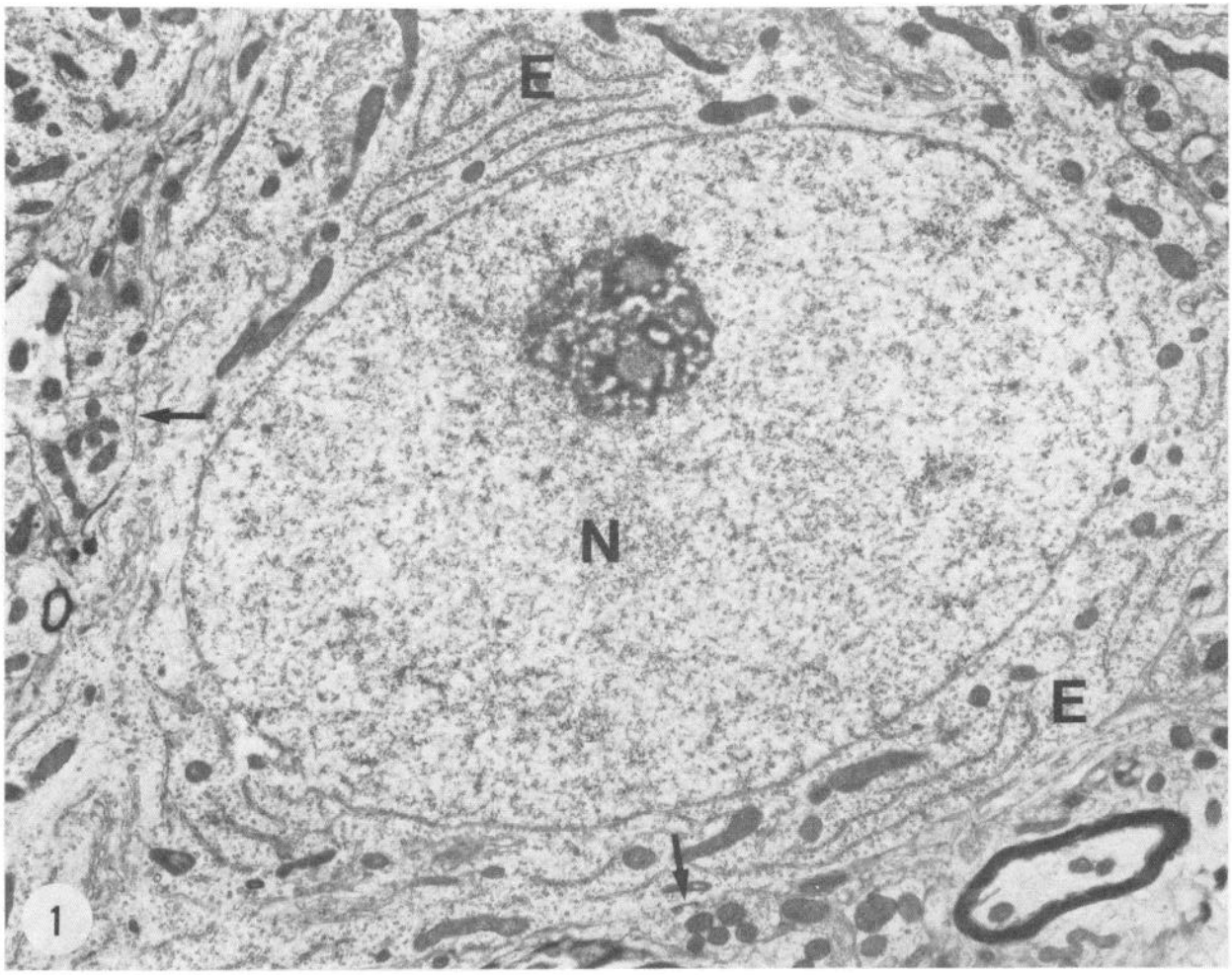
The following statistically significant changes were observed: (*1*) the number of axosomatic symmetric synapses with layer V pyramidal cells was decreased 80% at the focus and 50% at the parafocus, (*2*) in the neuropil adjacent to these pyramidal somata, the number of terminals forming symmetric synapses was reduced 50% at the epileptic focus but was unchanged at the parafocus, while the number of asymmetric synapses was reduced 25% at the focus and 15% at the parafocus, and (*3*) a 50% increase of glial profiles occurred at epileptic foci both in the neuropil and at sites apposed to pyramidal cell somata. The quantitative results also showed that terminals which form symmetric synapses had twice the number of mitochondria per terminal as those that form asymmetric synapses.

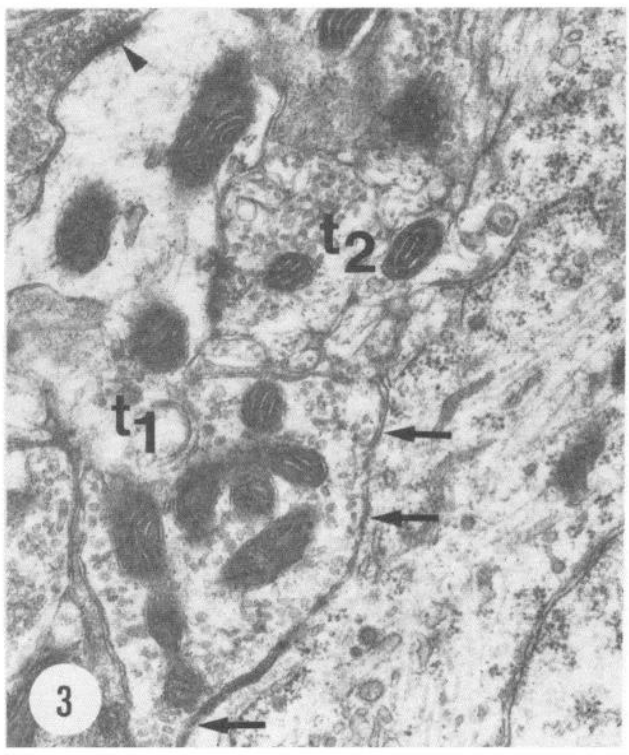
Axon terminals which form symmetric synapses with somata and dendrites in the neocortex have been shown previously to contain GAD. Therefore, the large reduction in the number of symmetric synapses at epileptic foci and the increased gliosis indicate that the previously observed loss of GABAergic terminals at sites of focal epilepsy is caused by terminal degeneration. Since such terminals are reduced more severely at epileptic foci than other terminals, their loss could be the basis for seizure activity due to a preferential decrease of inhibitory function at epileptic foci. Hypoxia has been shown to cause a selective degeneration of terminals with the same morphology as GABAergic terminals in the cortex, and the basis for this loss could be related to higher physiological and/or metabolic activities of GABAergic cortical cells which may inhibit other cells tonically. The fact that increased numbers of mitochondria occur in GABAergic terminals supports this idea.

Morphological changes associated with epilepsy can be studied directly in biopsies of human brains or in experimental animals with induced epilepsy. Procedures that have commonly been used to produce epileptic models include those: (*1*) induced by application of various agents, such as penicillin, cobalt, alumina gel, and kainic acid, and (*2*) induced by mimicry of human etiologies for

[1] This work was supported by Grants NS-15669, NS-04053, and NS-09037 from the National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health and a grant from the Epilepsy Foundation of America. We gratefully acknowledge Dr. Don S. Minckler, University of Southern California Medical School, for the generous use of the Zeiss Videoplan Computer, Dr. James E. Vaughn for valuable comments made on the manuscript, W. G. Kramer and L. Richman for technical assistance, and R. Enevold and N. Sepion for secretarial assistance.

[2] To whom correspondence should be addressed.





Figures 1–3

epilepsy, such as artificial postnatal hypoxia. Results obtained from studies that used these models suggest many significant morphological changes that differ from the normal. For instance, in an extensive morphological study of 25 monkeys made epileptic with alumina gel, Harris (1975) reported that extensive gliosis occurred at and adjacent to epileptogenic foci but that neuronal populations remained unchanged. Fischer (1969) also noted this increased gliosis in rats with cobalt-induced epilepsy, and, in addition, he reported that the cell bodies of cortical neurons were denuded of axosomatic symmetric synapses. Studies made of biopsy tissue from human patients with acute focal epilepsy also have shown this increased gliosis and loss of axosomatic symmetric synapses (Brown, 1973). In another human study, Williams et al. (1977) noted a conspicuous loss of symmetric synapses from the perikarya and axon hillocks of pyramidal cells in a 17-year-old patient with neuronal ceroid lipofuscinosis. More recently, Braak and Goebel (1978) reported that such patients have a decreased number of somata of aspinous stellate cells in cortical layers II and III. Sloper et al. (1980) showed degeneration of axon terminals making symmetric synapses in neonatal monkeys subjected to hypoxia. The reduction of these somata and terminals implies a loss of inhibitory function because: (*1*) these structures in the rat and monkey contain glutamic acid decarboxylase (GAD), the synthesizing enzyme for the neurotransmitter GABA (Ribak, 1978; Ribak et al., 1979), and (*2*) evidence from physiological and pharmacological studies indicates that GABA has an inhibitory action on cortical neurons (see Krnjević, 1974). Therefore, it is likely that seizures are facilitated by a loss of inhibitory cortical neurons.

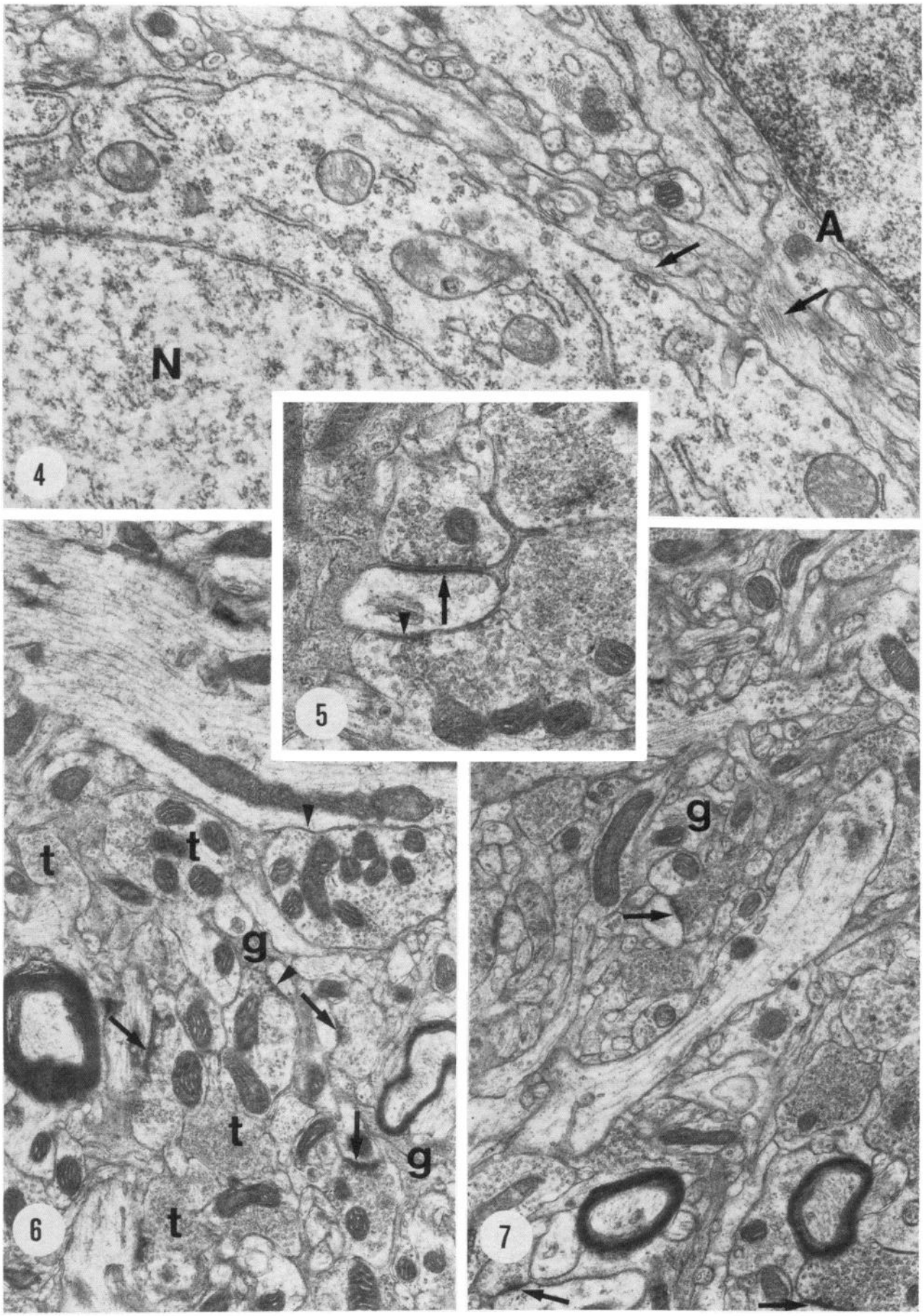
The alumina gel model of epilepsy is rated by many to be the most accurate model of human focal epilepsy. Subpial injections of alumina gel in monkeys produce a physiological activity in cortical neurons which is similar to the human epileptic condition (Wyler et al., 1978). This model generally is thought to be a better approximation of focal epilepsy in humans than other models because: (*1*) astrocytic gliosis and a slight neuronal depopulation at the focus are the same as that seen in human cortical scars (Harris, 1975; Ribak et al., 1981), (*2*) the seizures most often appear some months after the injections and, once established, may persist indefinitely in a way similar to the development of seizures in humans following trauma to the head, and (*3*) available anticonvulsant drugs which are effective in humans with seizures of cortical origin are also effective in monkeys with alumina gel-induced epilepsy (Purpura et al., 1972). Although previous ultrastructural studies have not analyzed alumina gel specimens for a loss of axosomatic symmetric synapses with pyramidal cells, recent results from immunocytochemical studies on this model of epilepsy suggest this possibility (Ribak et al., 1979). Preliminary electron microscopic observations add further support for the loss of axosomatic synapses in this model (Ribak et al., 1981).

Recent studies indicate that a reduction of GABA-mediated inhibition in the brain may cause focal epilepsy in these models as well as in humans. Balcar et al. (1978) have shown that, in cobalt-induced epilepsy, the kinetic parameters of GABA (V_{max} and K_m) are altered in such a way as to suggest a loss of GABA-mediated inhibition at the epileptogenic focus. Van Gelder and Courtois (1972) also have shown that cobalt-induced epilepsy in cats is characterized by a reduction in GABA levels at sites of focal epilepsy. In addition, these same authors have shown that epileptogenic human cortex has low levels of glutamic acid, a putative excitatory neurotransmitter which also serves as a precursor of GABA. A selective loss of GABA function in focal epilepsy has been questioned recently because Altamura et al. (1978) have shown large areas of random necrosis in the cobalt model. Thus, they suggested that the biochemical changes in levels of GABA and its synthesizing enzyme, GAD, are due to a general neuronal loss. However, recent biochemical studies of monkey and human cortical epileptic foci show reduced GAD activity, GABA concentration, and GABA receptor binding (Bakay and Harris, 1981; Lloyd et al., 1981). These latter studies are important because the markers for GABA function were affected more than markers for other neurotransmitters.

With immunocytochemical methods, Ribak and colleagues (1978, 1979) have shown in the cerebral cortex of rats and monkeys that GAD-positive reaction product is localized to the somata and terminals of the aspinous and sparsely spinous stellate neurons, an important subclass of local circuit neurons in the cerebral cortex (Peters and Fairén, 1978). The terminals of these neurons form only *symmetric* synapses on somata, dendrites, and axon initial segments of all cortical cells. In the electron microscope, the only terminals which contained GAD-positive reaction product were the ones forming symmetric synapses (Ribak, 1978; Ribak et al., 1979; Hendry et al., 1981). Furthermore, a prominent plexus of these terminals was found adjacent to the somata and proximal dendrites of pyramidal cells, thus coinciding with the pericellular baskets (Marin-Padilla, 1969; Ribak, 1978; Ribak et al., 1979; Emson and Hunt, 1981; Hendrickson et al., 1981; Jones, 1981). Therefore, GABAergic terminals are in a position to mediate a strong inhibition of projection neurons (pyramidal cells) in the neocortex due to the location and number of these GAD-positive ter-

Figure 1. Electron micrograph of a layer V pyramidal soma from a normal, nonepileptic hemisphere. The nucleus (*N*) of this soma contains a prominent nucleolus. The perikaryal cytoplasm has cisternae of endoplasmic reticulum (*E*) and other organelles typical of pyramidal cells. An apical dendrite is directed toward the *top* of the photomicrograph and a basal dendrite is partially shown in the *lower left corner*. Two terminals form axosomatic synapses (*arrows*) with this soma, while another synapse in this section is not shown. Magnification × 9,000.

Figures 2 and 3. Electron micrographs of the same soma as in Figure 1 but at a higher magnification to show axosomatic synapses. Figure 2 shows a small terminal (*t*) not illustrated in Figure 1. This terminal contains flattened synaptic vesicles and forms a symmetric synapse (*arrow*). Magnification × 32,000. Figure 3 shows the axosomatic terminal (t_1) on the *left side* of the soma in Figure 1. This terminal (t_1) contains many mitochondria and displays numerous active synaptic sites (*arrows*) where vesicles are grouped and membrane densities are apparent. In the field are two other terminals synapsing with a dendrite; one (t_2) forms a symmetric synapse and the other forms an asymmetric synapse (*arrowhead*). Magnification × 29,000.



Figures 4–7

minals. In monkeys made epileptic by cortical application of alumina gel, Ribak et al. (1979) found a significant decrease of GAD-positive puncta at seizure foci and in an area immediately surrounding it, the parafocus. Since previous data indicate that GAD-positive puncta with diameters of 1 to 2 μm are equivalent to axon terminals in the electron microscope (Ribak, 1978), these results suggest that a functional loss of GABAergic terminals occurs at sites of alumina gel application. This loss may be the basis for epileptic activity at these sites. However, results from this latter study did not determine whether the loss of inhibitory function coincided with a degeneration of GABAergic terminals or with a loss of GAD immunoreactivity within these terminals. In addition, it was not ascertained if this loss of GABAergic terminals was selective for this type of terminal. Information germane to the resolution of both of these issues can be obtained from ultrastructural studies with the use of quantitative methods. Therefore, the same monkey specimens used in the previous immunocytochemical study (Ribak et al., 1979) were examined in the present electron microscopic analysis.

Materials and Methods

The present study utilized specimens obtained from three of the five experimental monkeys from a previous study (Ribak et al., 1979). All of the monkeys had received alumina gel applications to the left cerebral hemispheres to produce seizure foci. Two of these monkeys (animals 3 and 5 from Ribak et al., 1979) received intracortical injections directly into both pre- and postcentral gyri, and the remaining experimental monkey (animal 2 from Ribak et al., 1979) had an injection of alumina gel limited to the subarachnoid space in the area of the central sulcus. In similar cases of alumina gel injected into the subarachnoid space, the alumina has been shown to remain as a granuloma in this space, although some alumina has been found in astrocytes in layer I, the most superficial part of the cortex (Harris, 1975). Electrocorticography of all experimental animals verified epileptic foci (Ribak et al., 1979), and subsequently, the monkeys were fixed by intracardiac perfusions of a mixture of two aldehydes. The fixative solution contained 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.002% $CaCl_2$ in a 0.12 M phosphate buffer.

Blocks of cortical tissue were obtained from the: (*1*) epileptic focus, (*2*) parafocus which exhibits epileptic activity following excision of the granuloma and primary focus (Harris and Lockard, 1981), and (*3*) homologous area in the contralateral nonepileptic cortex. Blocks of tissue from the parafocus were obtained from cortex 1 cm anterior or posterior to alumina gel-treated cortex. All specimens were sectioned on a Sorvall TC-2 tissue sectioner at a thickness of 150 μm and processed for electron microscopic observations by procedures detailed elsewhere (Ribak et al., 1979).

Quantitative study. The somata of 10 layer V pyramidal cells were selected randomly from each of the three sites for all three monkeys. These 90 somata were identified by their apical and basal dendrites, large size, and euchromatic nucleus (Fig. 1; Peters et al., 1976). Nonoverlapping portions of plasma membranes from these somata were photographed at a primary magnification of × 10,000. In addition, 2 or 3 electron micrographs at the same magnification were taken of the neuropil immediately adjacent to each pyramidal soma. All negatives were enlarged to a final print magnification of × 25,000. A total of 562 electron micrographs were analyzed for three specific structures that were each outlined with a different color ink: (*1*) axon terminals forming asymmetric synapses that displayed parallel pre- and postsynaptic membranes, relatively thick postsynaptic densities, and round vesicles accumulated at an active zone; (*2*) axon terminals forming symmetric synapses that displayed parallel pre- and postsynaptic membranes, symmetric pre- and postsynaptic densities, and elongated or flattened vesicles at active zones; and (*3*) glial profiles that were characterized by the presence of either numerous filaments, sparsely dispersed organelles, or cytoplasmic dense bodies corresponding to various types of lysosomes (Peters et al., 1976). An initial analysis was done blindly without knowing from which site the electron micrographs were obtained. Following this initial survey, each electron micrograph was analyzed again by another investigator to confirm the morphology of each circled profile using the same criteria. In most instances, these two analyses agreed remarkably well with each other.

Both the Zeiss Videoplan and Bioquant Apple II-Plus computer systems were used to measure the sizes of outlined profiles. Computer programs were selected to measure lengths and areas in values calibrated in micrometers. The following parameters were determined for the somata of pyramidal cells: (*1*) the length of each somal surface, (*2*) the length of the somal surface ap-

Figure 4. Electron micrograph showing a layer V pyramidal cell soma from an epileptic focus. The nucleus (*N*) is partially shown, and the somal surface is apposed by glial profiles that contain clusters of filaments typical of reactive astrocytes (*arrows*). Part of a soma of one of these astrocytes (*A*) is shown. Magnification × 27,000.

Figure 5. Electron micrograph showing, at a high magnification, an example of each terminal type in the cerebral cortex. The terminal which forms the asymmetric synapse (*arrow*) contains rounded vesicles. This synapse (*arrow*) is characterized by parallel pre- and postsynaptic membranes and a relatively large postsynaptic density. The other terminal forms a symmetric synapse (*arrowhead*) and contains flattened synaptic vesicles and numerous mitochondria. This synapse displays parallel pre- and postsynaptic membranes and a relatively small postsynaptic density. Magnification × 24,000.

Figure 6. Electron micrograph of a portion of neuropil adjacent to a layer V pyramidal neuron in nonepileptic cortex. Terminals which form asymmetric (*arrows*) and symmetric (*arrowheads*) synapses are shown. Other terminals (*t*) do not form synapses in the plane of this section. Profiles of glia (*g*) also are shown. Magnification × 18,000.

Figure 7. Electron micrograph of neuropil adjacent to a layer V pyramidal neuron in an epileptic focus. Terminals which form asymmetric synapses (*arrows*) are shown, but those which form symmetric synapses are not present. Profiles of glia (*g*) also are present. Computer analysis of these and other electron micrographs revealed quantitative differences between nonepileptic cortex and the parafocus and focus. Magnification × 18,000.

posed by terminals that form symmetric axosomatic synapses, (3) the area of these terminals, (4) the length of the somal surface apposed by glia, and (5) the area of these glial profiles. The total length of the somal surface measured from these electron micrographs was 4,550 μm. The following measurements were made for the neuropil adjacent to each soma: (1) total area of analyzed neuropil, (2) the area of terminals that form asymmetric synapses in this field, (3) the area of terminals that form symmetric synapses, and (4) the area of glial profiles in this field. The total area of the neuropil was 12,900 μm^2. In addition, the number of mitochondria were counted in each terminal observed in the micrographs.

These raw data were used to obtain the following parameters: (1) the number of terminals forming axosomatic symmetric synapses per unit length (10 μm) of somal surface for layer V pyramidal cells, (2) the relative amounts of somal surface apposed by terminals and glia, (3) the percentage of neuropil area occupied by terminals that form asymmetric and symmetric synapses, (4) the relative amount of neuropil occupied by glia, (5) the areas of terminals in all micrographs, and (6) the number of asymmetric and symmetric synapses per unit area (10 μm^2) of neuropil. For this last parameter, the total area of all glial profiles was subtracted from the total area of the neuropil so that this parameter would reflect only variations between types of synaptic profiles and not the variations that are due to fluctuations in the amount of glia. Paired *t* tests were done between the resulting data groups to determine the probability that these parameters were obtained from significantly different populations (Sokal and Rohlf, 1969).

Results

A general survey of the entire depth of the cerebral cortex was made from layer I to the white matter in electron microscopic preparations. The types of cells observed were consistent with those previously described by Sloper (1973) for the primate sensorimotor cortex, and these included both pyramidal and stellate types. In addition, the various types of dendrites, terminals, and synapses described by Sloper (1973) were found. The detailed analyses of the present study were focused on pyramidal neurons in layer V because: (1) our previous GAD immunocytochemical study was made in this layer (Ribak et al., 1979) and (2) pyramidal neurons of this layer are known to be the origin of the majority of cortical and subcortical projections (Jones, 1981).

Nonepileptic cortex. Layer V pyramidal neurons were identified in electron microscopic preparations of nonepileptic specimens, and they displayed characteristics that have been described previously (Sloper, 1973; Peters et al., 1976; Gatter et al., 1978). Typically, layer V pyramidal neurons are larger than other cortical cells, and the somata have multiangular shapes formed by an array of basal dendrites, a single apical dendrite, and a single axon usually located between the basal dendrites. Much of the perikaryon is occupied by a large, rounded nucleus whose nucleoplasm contains a relatively homogeneous sprinkling of electron-opaque chromatin and perichromatin masses (Fig. 1). In addition to the more common cytoplasmic features, such as mitochondria, Golgi apparatus, and microtubules, layer V pyramidal neurons contain a typical pattern of both free ribosomes and cisternae of granular endoplasmic reticulum that form Nissl bodies (Fig. 1). The terminals that form axosomatic synapses with these neurons make only symmetric synapses as previously described (Sloper, 1973; Peters et al., 1976; Gatter et al., 1978; Colonnier, 1981). The synaptic complexes of these terminals have presynaptic densities resulting from an accumulation of dense particles at the cytoplasmic face of the presynaptic membrane, a synaptic cleft (20 to 30 nm wide) containing a small amount of electron-dense material, and a postsynaptic density, similar in thickness to the presynaptic density, caused by an accumulation of dense material at the inner face of the somal plasma membrane (Figs. 2 and 3). The vesicles within these terminals have elongated or flattened shapes.

The neuropil adjacent to layer V pyramidal neurons displays a variety of components, including terminals which form symmetric synapses, terminals which form asymmetric synapses, dendrites, axons, and astrocytic processes (Figs. 5 to 7). Terminals that form asymmetric synapses do so with dendritic shafts and spines in the neuropil and with the somata of aspinous and sparsely spinous stellate cells. The axoplasm of asymmetric terminals contains numerous rounded vesicles and occasional mitochondria (Fig. 5). The synaptic complexes associated with these terminals have presynaptic densities and synaptic clefts like those of terminals that form symmetric synapses. However, an increased thickness of the postsynaptic dense material occurs at asymmetric synapses (Fig. 5). Dendrites contain mitochondria, clusters of ribosomes, and parallelly-oriented microtubules. Axons, like dendrites, contain microtubules and mitochondria; however, they lack ribosomes, occasionally are surrounded by thick myelin sheaths, and sometimes have synaptic vesicles scattered through their axoplasm. The processes of protoplasmic astrocytes are observed adjacent to pyramidal cell somata and in the neuropil. Typically, these processes have irregular shapes which conform to the contours of the comparatively uniform shapes of axons, axon terminals, dendrites, and perikaryal membranes (Peters et al., 1976). In nonepileptic cortex, these processes generally lack cytoplasmic filaments. Using these criteria, neuronal and glial profiles were identified for the quantitative ultrastructural study.

Epileptic cortex. Thin sections of cortical tissue from both the epileptic focus and the parafocus contained two morphologically distinct differences as compared with nonepileptic cortex. First, there was a noticeable decrease in the number of terminals that form symmetric, axosomatic synapses with the somata of pyramidal neurons in layer V (Fig. 4). For example, the number of symmetric synapses per soma of layer V pyramidal neuron in nonepileptic preparations ranged from 1 to 17 synapses for the 30 cells counted, while the 30 somata studied from epileptic foci had from 0 to 3 synapses per soma, and 12 of these latter somata lacked synapses. The somata studied in the parafocus ranged from 0 to 9 synapses per soma. Similar observations also were made for somata located in the other cortical layers. The second difference was an increased number of astrocytic processes both in the neuropil and adjacent to pyramidal cell somata, and these processes contained numerous filaments (Fig. 4).

TABLE I

Comparative data for the three monkeys (ME2, ME3, and ME5) used in the ultrastructural quantitative study

Data are shown for the three examined sites: the normal, nonepileptic hemisphere (N), the parafocus (P), and the focus (F). The GAD immunocytochemistry data for each monkey were obtained from Ribak et al. (1979) and are expressed as the mean number of GAD-positive axon terminals per 3,000 μm^3 of tissue in layers V and VI of monkey sensorimotor cortex. The average number of terminals per 10 μm of somal surface is referred to as "EM axosomatic," while the mean number of terminals which form symmetric synapses per 10 μm^2 of neuropil is labeled "EM neuropil." These latter data have means which are less than the ones expressed in the text because the glial areas were not omitted from the total neuropil areas for the data in the table. The average percentage of loss at the focus and parafocus is on the right.

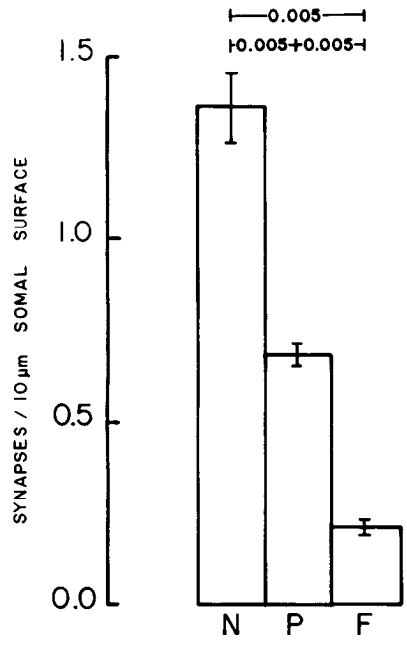
	Data			Loss	
	N	P	F	F	P
				%	
ME2					
GAD immunocytochemistry	17.2	13.5	6.7	61.0	21.5
EM axosomatic	1.52	0.67	0.31	79.6	55.9
EM neuropil	0.28	0.27	0.10	64.0	3.60
ME3					
GAD immunocytochemistry	11.6	8.2	4.4	62.1	29.3
EM axosomatic	1.25	0.63	0.16	87.2	49.6
EM neuropil	0.23	0.24	0.16	30.4	4.3[a]
ME5					
GAD immunocytochemistry	23.6	15.8	10.0	57.6	33.0
EM axosomatic	1.19	0.75	0.30	74.7	36.9
EM neuropil	0.17	0.20	0.06	64.7	17.6[a]

[a] Percentage of increase.

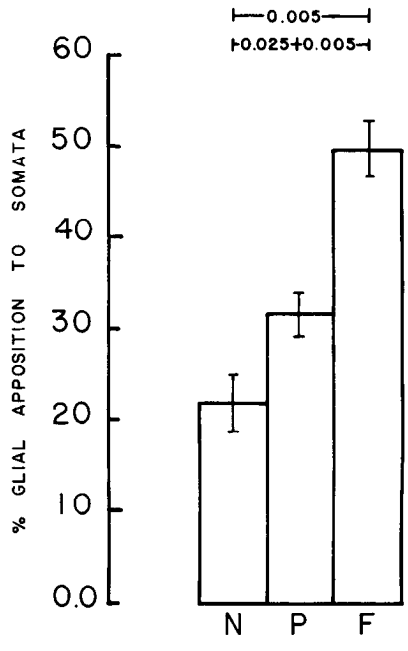
No other striking differences were observed in these initial observations. The ultrastructural characteristics of neuronal somata in epileptic foci appeared similar to those in normal cortex. In addition, preparations of neuropil from foci and parafoci were similar to the preparations of nonepileptic neuropil (Figs. 6 and 7).

Results of quantitative analysis. The data obtained from the parametric analyses confirmed all of the initial qualitative observations and indicated additional morphological differences. As shown in Table I, the number of terminals per unit length of somal surface was greatest in normal cortex and least at the epileptic focus for all three monkeys. Averaging the values presented for individual specimens in Table I revealed that the number of axosomatic terminals decreased from 1.32 per unit length of pyramidal cell somal surface in normal cortex to 0.68 per unit length in the parafocus to 0.28 per unit length in the focus (Fig. 8). Thus, the focus displayed an 80% loss of these terminals, while the parafocus showed only a 50% loss. All of these differences proved to be highly significant ($p < 0.005$).

An alternative method for expressing these results was utilized to take into account possible changes in the sizes of the neuronal somata and axosomatic terminals within the focus and parafocus resulting from alumina gel application. The length of somal membrane of layer V pyramidal cells occupied by synapsing axon terminals was calculated, and this figure was expressed as a percentage for each of the three sites. The combined average percentage (for the three monkeys) of somal surface occupied by terminals was 17.5% in normal cortex, 10.1% in the parafocus, and 2.7% in the focus. Thus, the per-



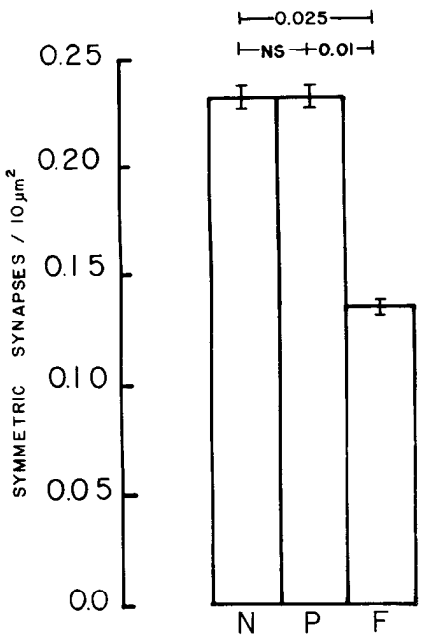
8



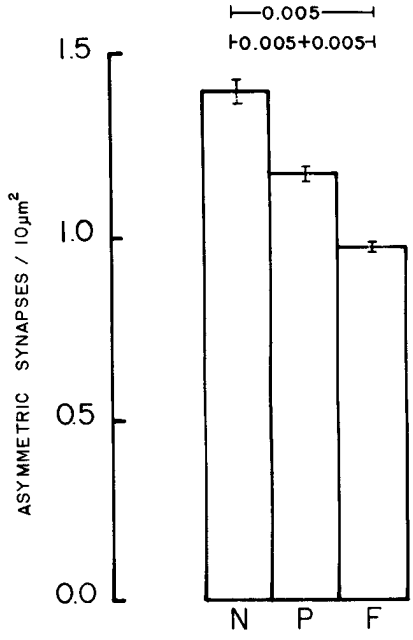
9

Figure 8. Bar graph showing the number of synapses per 10 μm of somal surface of 90 layer V pyramidal somata. Ten cells from each site of each of three monkeys were counted. The number of these synapses was greatest in the normal nonepileptic cortex (*N*) and least at the focus (*F*). The parafocus (*P*) had an intermediate number. In this and the following figures, mean values were compared by the Student's *t* test. Significant differences are indicated by probability levels ($p <$ indicated value) and no significant differences are indicated by NS. The standard error of the mean appears at the top of each *bar*.

Figure 9. Bar graph showing the highly significant increases in glial apposition to layer V pyramidal neurons at the parafocus (*P*) and focus (*F*). *N* indicates the normal nonepileptic cortex.



10



11

Figure 10. Bar graph showing the number of terminals per 10 μm^2 which form symmetric synapses in the neuropil adjacent to layer V pyramidal cells. The focus (*F*) showed a significant difference with both the parafocus (*P*) and normal cortex (*N*). No significant differences are indicated by *NS*.

Figure 11. Bar graph showing decreases in the number of terminals forming asymmetric synapses in the neuropil of the parafocus (*P*) and focus (*F*). These changes were significant, but the percentages of change were not as great as those for the axosomatic synapses (see Fig. 8). *N* indicates the normal cortex.

centages of terminal loss at the focus and parafocus as compared to normal cortex were 85% and 42%, respectively. These values are similar to the ones obtained by the first method (see above; 80% and 50%, respectively). In summary, the two methods used to evaluate the number of axosomatic synapses for layer V pyramidal cells have both shown that a dramatic loss of these synapses occurs at epileptic foci.

Measurements of the length of somal surface apposed by glial processes also were made, and these data are represented as percentages of total somal surface in Figure 9. The results showed significant increases in glial apposition in the parafocus and highly significant increases in the focus. These data essentially reflect the descriptive findings which showed a loss of axosomatic terminals and an increase in glial apposition to the somata of layer V pyramidal neurons in epileptic cortex, both focus and parafocus.

Differences in the number of neuropil components in the focus and parafocus as compared to normal cortex were not obvious initially but were revealed by a parametric analysis of the electron microscopic data. Measurements of the total area of the neuropil and the areas occupied by terminals that form either asymmetric or symmetric synapses yielded results expressed as the number of terminals per 10 μm^2 of neuropil (Table I; Figs. 10 and 11). The average values obtained for all three animals showed that terminals forming asymmetric synapses were decreased significantly from 1.36 synapses per unit area in nonepileptic cortex to 1.21 synapses per unit area in the parafocus and to 0.97 synapses per unit area in epileptic, focal cortex ($p < 0.005$). Thus, the focus had about a 25% loss of terminals which form asymmetric synapses when compared with nonepileptic cortex, and the parafocus had a 15% loss (Fig. 11). These two sites also were significantly different from each other ($p < 0.005$). Terminals which form symmetric synapses were less frequent in the neuropil than those that form asymmetric synapses (compare the ordinates for Figs. 10 and 11). The frequency of the symmetric terminals also showed statistically significant differences in that the focus had 0.13 synapses per unit area and the parafocus had 0.23 synapses per unit area ($p < 0.01$). Significant differences also were found between the focus and nonepileptic cortex ($p < 0.025$). However, the parafocus and nonepileptic cortex did not display any significant differences in the frequency of terminals forming symmetric synapses in the neuropil. In addition, measurements made on glial profiles showed a 50% increase at the focus ($p < 0.001$) and a 30% increase at the parafocus ($p < 0.001$).

The only statistically significant data for the mean area (size) of individual terminals showed that the axosomatic terminals were larger ($p < 0.025$) than the terminals which form asymmetric synapses in the neuropil (Fig. 12). The axosomatic synapses were also slightly larger than the terminals forming symmetric synapses in the neuropil (Fig. 12). These size differences between terminals forming symmetric and asymmetric synapses were observed in all three cortical sites, but the average size of the terminal types did not differ significantly among the three different cortical sites (normal cortex, parafocus, and focus). A total of 2,034 terminals were measured in this study, and Table II shows the distribution by cortical site and type of synapse. The data on mitochondria in terminals showed that both types of

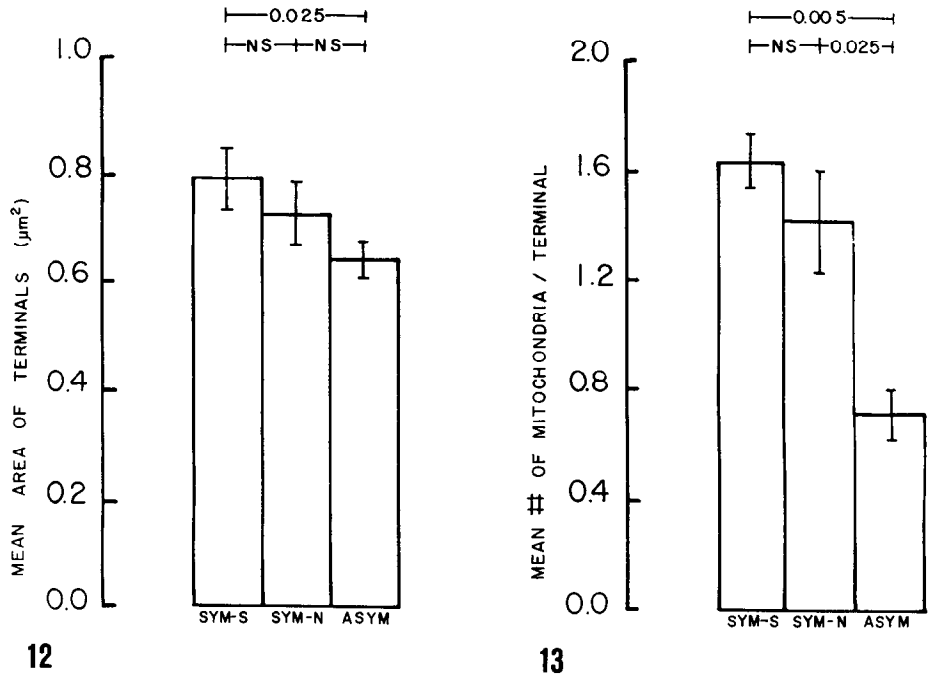


Figure 12. Bar graph showing the mean area (size) of all terminals counted in this study. The terminals forming symmetric synapses with somata (*SYM-S*) have the largest area, while terminals forming symmetric synapses in the neuropil (*SYM-N*) were intermediate in size. The only statistically significant decrease was between the axosomatic terminals and those forming asymmetric synapses (*ASYM*). No significant differences are indicated by *NS*.

Figure 13. Bar graph showing the mean number of mitochondria per terminal for each of the same categories in Figure 12. The terminals which form symmetric synapses contained more than twice the number of mitochondria found in the terminals that form asymmetric synapses.

TABLE II

Number of terminals measured

A total of 2,034 terminals were measured for this analysis. They are divided in this table into two categories, type and cortical site. Three types of terminals were analyzed and they included those terminals that form: (*1*) asymmetric synapses, (*2*) symmetric synapses in the neuropil, and (*3*) symmetric, axosomatic synapses with layer V pyramidal somata. The three cortical sites included the normal cortex, the parafocus, and the focus (see the text for details). The data for the parafocus reflect a slightly larger area of measured neuropil for this site.

Type	Cortical Site		
	Normal	Parafocus	Focus
Asymmetric	562	534	393
Symmetric in the neuropil	82	109	42
Symmetric axosomatic	169	118	25

terminals which form symmetric synapses each had more than twice the mean number of mitochondria per terminal than the type of terminal that forms asymmetric synapses (Fig. 13) had. These differences were statistically significant. It is interesting to note that the terminals which form axosomatic synapses had slightly more mitochondria in their larger terminals than the ones forming symmetric synapses in the neuropil (Fig. 13).

Discussion

In a previous study, light microscopic preparations of monkey cortex incubated in anti-GAD serum revealed that a severe loss of GAD-positive terminals occurred at sites of alumina gel-induced epilepsy (Ribak et al., 1979). These results suggested that this loss was due to either a degeneration of terminals containing GAD (GAD-positive terminals) or a loss of GAD immunoreactivity within GABAergic terminals. The present results show a significant loss of terminals forming axosomatic symmetric synapses at both the focus and parafocus. Although symmetric synapses in the neuropil were reduced only in the focus, the combined data on the percentage of loss for both types of symmetric synapses compare favorably with the percentage of loss of GAD-positive terminals for the same monkey specimens (Table I). Since terminals that form symmetric synapses in the neocortex are very likely to contain the GABA-synthesizing enzyme, GAD (Ribak, 1978; Ribak et al., 1979), these results indicate that the observed loss of GAD-positive terminals at epileptic foci is due to the degeneration of terminals derived from GABAergic cortical cells. The large loss of these GABAergic terminals indicates a significant reduction of inhibition at epileptic foci because GABA has an inhibitory action on cortical neurons (Krnjević, 1974). Thus, a probable result of this loss is a hyperexcitability of neurons in the focus.

Degeneration of neurons in Fink-Heimer preparations has been reported previously for alumina gel-treated, epileptic monkeys (Harris, 1972). A common sequel to degeneration in the central nervous system is the hypertrophy and proliferation of glia (Peters et al., 1976). Increased gliosis at epileptic foci was demonstrated for this model of epilepsy (Harris, 1975) as well as for other models (Fischer, 1969; Brown, 1973; Williams et al., 1977). The results of the present study showed a 50% increase

of glial profiles at epileptic foci both in the neuropil and at sites apposed to pyramidal cell somata. These data are consistent with previous results that showed glial proliferation at epileptic foci and indicated degeneration of neurons. A likely cause of this proliferation in the alumina gel model of epilepsy is the significant loss of GABAergic axon terminals.

A severe loss of GABAergic inhibitory function, such as that which occurs in alumina gel-induced focal epilepsy, could explain seizure activity if the magnitude of this loss was greater than that of the other cortical terminal type that is thought to be associated with excitatory function. In the cerebral cortex, this second type of terminal forms asymmetric synapses and occurs more frequently as evidenced by the data which show that asymmetric synapses are 7 times more abundant than symmetric synapses in the neuropil (Figs. 10 and 11). Results of electron microscopic studies of physiologically identified axons in the cerebral cortex have shown that the terminals which form asymmetric synapses are likely to be excitatory (see Colonnier, 1981). For example, the axons of pyramidal cells are considered to be the excitatory output from the neocortex, and they have terminals that form asymmetric synapses. The present results indicate that a significant loss of terminals that form asymmetric synapses occurs at both the focus and parafocus. However, the loss of these terminals at epileptic foci (25%) is much less than the more severe loss of terminals that form symmetric synapses adjacent to pyramidal cell somata (80%) and in the neuropil (50%). The loss of terminals forming asymmetric synapses at the parafocus was smaller than that at the focus. In a way similar to the focus, this loss of asymmetric synapses at the parafocus (15%) is substantially less than the 50% loss of axosomatic symmetric synapses. In contrast, the frequency of the other category of symmetric synapse (axodendritic) in the parafocus was not significantly different from that in normal cortex. Nevertheless, the magnitude of the axosomatic GABAergic terminal loss is greater at epileptic foci than that of the excitatory terminal type. The location of this terminal loss upon the soma and in close proximity to the axon hillock of pyramidal cells undoubtedly removes a large portion of probably the most effective inhibitory control of cortical projection neurons.

The cause of a certain amount of terminal loss is probably the general degeneration from direct trauma of the procedure of alumina gel application. For example, many pyramidal cells which have axons forming asymmetric synapses and corticocortical connections (Jones, 1981) in the focus and parafocus probably are destroyed by the alumina gel granuloma. Thus, many terminals of this type would be expected to degenerate. However, the larger percentage of loss of symmetric synapses is more of a quandary since the aspinous and sparsely spinous stellate cells which form these synapses (Peters and Fairén, 1978) are strictly local circuit neurons with much shorter axons that do not make corticocortical connections. Thus, an additional mechanism would seem to be necessary to explain the increased loss of GABAergic terminals at epileptic foci.

Possible causes of the preferential loss of GABAergic terminals at epileptic foci. The reasons for the increased loss of GABAergic terminals in this model are unknown. However, it is possible that the cortical local circuit neurons that give rise to these terminals may be more sensitive to the effects of alumina gel application than the putative excitatory terminals that form asymmetric synapses. Sloper et al. (1980) have shown in neonatal monkeys subjected to 30 min of severe hypoxia that terminals with the same morphology of GABAergic terminals degenerate and that this degeneration is most severe in layer V of the cortex. These authors note that one of the most striking ultrastructural features of local circuit neurons which give rise to these terminals is the large number of mitochondria in their cytoplasm. This finding has been confirmed in our quantitative study which shows that the mean sizes of cortical terminals do not vary greatly but that the mean number of mitochondria per terminal forming *symmetric* synapses was more than twice the number of mitochondria per terminal forming *asymmetric* synapses. These data suggest that GABAergic terminals which form symmetric synapses have a higher reliance on aerobic metabolism than the other cortical terminals which form asymmetric synapses and are thought to be associated with excitatory transmission. Since much of the energy-dependent activities of axon terminals is related to synaptic transmission (see "Review" in Peters et al., 1976), the increased number of mitochondria in GABAergic terminals may mean that these terminals release neurotransmitter at more frequent intervals than the excitatory cortical terminals. The fact that the axosomatic symmetric terminals had a larger number of mitochondria per terminal than the terminals which form symmetric synapses in the neuropil (Fig. 13) suggests that the most "active" terminals are from the cell type that sends its axons to somata, the basket cells (Marin-Padilla, 1969; Jones, 1981). This is consistent with the idea that the GABAergic, pericellular basket plexus that forms axosomatic symmetric synapses may provide a tonically active inhibition of cortical projection neurons (Roberts, 1980).

If, as suggested above, GABAergic neurons are more dependent upon aerobic metabolism than other types of cortical neurons, they may be affected more severely by topically administered alumina gel to the surface of the cortex because the functioning of the cortical vasculature which arises from the cortical surface may be compromised by this treatment. Therefore, the preferential loss of GABAergic terminals in both the alumina gel model and the artificial postnatal hypoxia model might be caused by a greater susceptibility of GABAergic, cortical neurons to hypoxic conditions. Since basket cell terminals appear to be affected most severely in the alumina model, a pathological hierarchy might exist between basket cells whose axons form axosomatic synapses and other cortical GABA cell types whose axons form axodendritic synapses, and this could reflect a functional hierarchy of GABAergic, cortical cells related to a cell's firing and/or metabolic rate. A hypoxia hypothesis agrees well with the results of other investigations which have shown that the incidence of epilepsy in rodents increases after being subjected to hypoxic conditions or conditions of restricted blood supply (Kalimo et al., 1979; Brown et

al., 1979). Thus, hypoxia may result in an accelerated death of GABAergic stellate neurons which, in turn, could be expected to reduce the inhibitory synaptic control over pyramidal neurons and lead to seizure activity.

References

Altamura, A. C., M. Bonati, N. Brunello, P. L. Giordano, and S. Algeri (1978) The activity of some neurotransmitter-synthesizing enzymes in experimental cobalt epilepsy. Neurosci. Lett. *7:* 83–87.

Bakay, R. A. E., and A. B. Harris (1981) Neurotransmitter, receptor and biochemical changes in monkey cortical epileptic foci. Brain Res. *206:* 387–404.

Balcar, V. J., R. Pumain, J. Mark, J. Borg, and P. Mandel (1978) GABA-mediated inhibition in the epileptogenic focus, a process which may be involved in the mechanism of cobalt induced epilepsy. Brain Res. *154:* 182–185.

Braak, H., and H. H. Goebel (1978) Loss of pigment-laden stellate cells: A severe alteration of the isocortex in juvenile neuronal ceroid-lipofuscinosis. Acta Neuropathol. (Berl.) *42:* 53–57.

Brown, A. W., D. E. Levy, M. Kublick, J. Hannow, F. Plum, and J. B. Brierley (1979) Selective chromatolysis of neurons in gerbil brain: A possible consequence of "epileptic" activity produced by common carotid artery occlusion. Ann. Neurol. *5:* 127–138.

Brown, W. J. (1973) Structural substrates of seizure foci in the human temporal lobe. In *Epilepsy, Its Phenomena in Man,* M. A. B. Brazier, ed., pp. 339–374, Academic Press, New York.

Colonnier, M. (1981) The electron-microscopic analysis of the neuronal organization of the cerebral cortex. In *The Organization of the Cerebral Cortex,* F. O. Schmitt, F. G. Worden, G. Adelman, and S. G. Dennis, eds., pp. 125–152, M. I. T. Press, Cambridge, MA.

Emson, P. C., and S. P. Hunt (1981) Anatomical chemistry of the cerebral cortex. In *The Organization of the Cerebral Cortex,* F. O. Schmidt, F. G. Worden, G. Adelman, and S. G. Dennis, eds., pp. 325–345, M. I. T. Press, Cambridge, MA.

Fischer, J. (1969) Electron microscopic alterations in the vicinity of epileptogenic cobalt-gelatine necrosis in the cerebral cortex of the rat. Acta Neuropathol. (Berl.) *14:* 201–214.

Gatter, K. C., J. J. Sloper, and T. P. S. Powell (1978) An electron microscopic study of the termination of intracortical axons upon Betz cells in area 4 of the monkey. Brain *101:* 543–553.

Harris, A. B. (1972) Degeneration in experimental epileptic foci. Arch. Neurol. *26:* 434–449.

Harris, A. B. (1975) Cortical neuroglia in experimental epilepsy. Exp. Neurol. *49:* 691–715.

Harris, A. B., and J. S. Lockard (1981) Absence of seizures or mirror foci in experimental epilepsy after excision of alumina and astrogliotic scar. Epilepsia *22:* 107–122.

Hendrickson, A. E., S. P. Hunt, and J. -Y. Wu (1981) Immunocytochemical localization of glutamic acid decarboxylase in monkey striate cortex. Nature *292:* 605–607.

Hendry, S. H. C., C. R. Houser, E. G. Jones, and J. E. Vaughn (1981) Synaptic relations of GABAergic intrinsic neurons in monkey somatic sensory cortex. Soc. Neurosci. Abstr. *7:* 833.

Jones, E. G. (1981) Anatomy of cerebral cortex: Columnar input-output organization. In *The Organization of the Cerebral Cortex,* F. O. Schmitt, F. G. Worden, G. Adelman, and S. G. Dennis, eds., pp. 199–235, M. I. T. Press, Cambridge, MA.

Kalimo, H., L. Paljarvi, and M. Vapalahti (1979) The early ultrastructural alterations in the rabbit cerebral cortex after compression ischemia. Neuropathol. Appl. Neurobiol. *5:* 211–223.

Krnjević, K. (1974) Chemical nature of synaptic transmission in vertebrates. Physiol. Rev. *54:* 418–540.

Lloyd, K. G., C. Munari, L. Bossi, J. Bancaud, J. Talairach, and P. L. Morselli (1981) Biochemical evidence for the alterations of GABA-mediated synaptic transmission in human epileptic foci. In *Neurotransmitters, Seizures and Epilepsy,* P. L. Morselli, K. G. Lloyd, W. Löscher, B. Meldrum, and E. H. Reynolds, eds., pp. 331–354, Raven Press, New York.

Marin-Padilla, M. (1969) Origin of pericellular baskets of the human motor cortex: A Golgi study. Brain Res. *14:* 633–646.

Peters, A., and A. Fairén (1978) Smooth and sparsely-spined stellate cells in the visual cortex of the rat: A study using a combined Golgi-electron microscopic technique. J. Comp. Neurol. *181:* 129–171.

Peters, A., S. L. Palay, and H. deF. Webster (1976) *The Fine Structure of the Nervous System: The Neurons and Supporting Cells,* W. B. Saunders Co., Philadelphia.

Purpura, D. P., J. K. Peury, D. B. Tower, D. M. Woodward, and R. D. Walter (1972) *Experimental Models of Epilepsy—A Manual for the Laboratory Worker,* Raven Press, New York.

Ribak, C. E. (1978) Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase. J. Neurocytol. *7:* 461–478.

Ribak, C. E., A. B. Harris, J. E. Vaughn, and E. Roberts (1979) Inhibitory, GABAergic nerve terminals decrease at sites of focal epilepsy. Science *205:* 211–214.

Ribak, C. E., A. B. Harris, J. E. Vaughn, and E. Roberts (1981) Immunocytochemical changes in cortical, GABA neurons in a monkey model of epilepsy. In *Neurotransmitters, Seizures and Epilepsy,* P. L. Morselli, K. G. Lloyd, W. Löscher, B. Meldrum, and E. H. Reynolds, eds., pp. 11–22, Raven Press, New York.

Roberts, E. (1980) Epilepsy and antiepileptic drugs: A speculative synthesis. In *Antiepileptic Drugs: Mechanisms of Action,* G. H. Glaser, J. K. Penry, and D. M. Woodbury, eds., pp. 667–713, Raven Press, New York.

Sloper, J. J. (1973) An electron microscopic study of the neurons of the primate motor and somatic sensory cortices. J. Neurocytol. *2:* 351–359.

Sloper, J. J., P. Johnson, and T. P. S. Powell (1980) Selective degeneration of interneurons in the motor cortex of infant monkeys following controlled hypoxia: A possible cause of epilepsy. Brain Res. *198:* 204–209.

Sokal, R. R., and J. Rohlf (1969) *Biometry,* W. H. Freeman and Co., San Francisco.

Van Gelder, N. M., and A. Courtois (1972) Close correlation between changing content of specific amino acids in epileptogenic cortex of cats, and severity of epilepsy. Brain Res. *43:* 477–484.

Williams, R. S., I. T. Lett, R. J. Ferrante, and V. S. Caviness, Jr. (1977) The cellular pathology of neuronal ceroid lipofuscinosis. Arch. Neurol. *34:* 298–305.

Wyler, A. R., K. J. Burchiel, and A. A. Ward, Jr. (1978) Chronic epileptic foci in monkeys: Correlation between seizure frequency and proportion of pacemaker epileptic neurons. Epilepsia *19:* 475–483.